Calcium and Repression in Malaria Sex: Knowing When the Time Is Right

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Translational repression is important for development of the malaria parasite when establishing infection in the mosquito. In this issue of *Cell Host and Microbe*, Sebastian et al. (2012) show that a calcium-dependent protein kinase is important for alleviating translational repression during developmental progression.

Pathogenic organisms' life cycles can be extremely complex, often requiring rapid changes in host and tissue tropism for their completion. The demand this places on parasites is compounded by a need to meet the threat of host immune responses, meaning parasites must be capable of significant biological gymnastics. The success of *Plasmodium* species, agents of malaria, and the cause of nearly 1 million deaths annually, relies on the ability to circumvent recognition by the human immune system and then rapidly differentiate when taken up by an *Anopheles* mosquito. Indeed, transmission from the human to mosquito host is the biggest population bottleneck in the *Plasmodium* life cycle (Sinden and Billingsley, 2001) and is therefore touted as a promising target to break the cycle of malaria transmission. Preparation for transmission occurs during the asexual replicative cycle, where a proportion of parasites differentiate into the sexual-stage gametocytes. After development for over a week, these are ready to be taken up in the mosquito blood meal and differentiate into male and female gametes in the midgut. Gamete fertilization then forms a zygote that rapidly develops into the motile ookinete. The ookinete escapes the midgut into the mosquito circulatory system, thanks to the activity of an actomyosin motility motor that allows the parasite to actively penetrate the midgut wall.

One method that is thought to help *Plasmodium* deal with the challenges faced during this host transition is translational repression (TR). Here, transcripts required for gamete fertilization and ookinete development are quiescently stored in so-called P granules, in the female gametocyte. mRNA storage is dependent on the RNA helicase DOZI and an Sm-like factor, called CITH (Mair et al., 2006, 2010). As in metazoans, repression of these transcripts is relieved following gamete fertilization. The reason *Plasmodium* stores transcripts for translation at a later date is not known, though it is clear that TR is vital for infection of the mosquito host. One possibility is that TR is needed for quick adaptation from growth in the human to the mosquito host, whereby development of the ookinete can occur much faster by removing the need for transcription from the newly formed zygote genome. This means that vulnerable zygotes aren't lingering in the hostile midgut environment longer than needed. It is also possible that TR is important for suppressing premature antigen expression, thereby avoiding production of antisexual-stage antibodies in the human host. Indeed, antibodies to ookinete surface proteins are known to block mosquito infection if taken up in the blood meal, offering the exciting prospect that these could make transmission blocking vaccines (Saul, 2007).

How do *Plasmodium* parasites relieve TR in the developing zygote? Sebastian et al. (2012) provide evidence that calcium-mediated signaling pathways might be the key. Here, they demonstrate that a calcium-dependent protein kinase (CDPK1) that was previously implicated in parasite motility and invasion in fact controls the alleviation of TR upon gamete fertilization. This is the first indication of how TR is regulated in *Plasmodium* and hints at a new area of exciting biology.

Calcium-signaling events in *Plasmodium* are known to regulate a range of cellular processes, including red blood cell invasion during disease (Billker et al., 2009). Although very little is known about the molecular mechanics underlying these pathways, there is growing evidence that a range of CDPKs are key effectors of calcium signaling. CDPKs were first described as vital components of a slew of signaling pathways in plants before their curious identification in certain groups of protists—including the phylum Apicomplexa, to which *Plasmodium* belongs. These unusual kinases represent a direct fusion between a kinase domain and a calcium-binding calmodulin-like domain (Billker et al., 2009). CDPKs are therefore able to directly translate calcium flux into enzymatic activity, without activation by other proteins. This is a mechanism unlike anything found in mammalian systems, making CDPKs of significant interest as drug targets.

CDPK1 has been of particular interest in a search for a drug target in blood stage infections, as it has been suggested to be a key regulator of the actomyosin motor during red blood cell invasion. In *Plasmodium falciparum* blood stages, *Pf*CDPK1 is coexpressed with a range of genes involved in parasite motility and invasion, and in vitro data has shown *Pf*CDPK1 phosphorylates gliding-associated protein 45 (GAP45) and MyoA-tail interacting protein (MTIP) of the motor complex in a calcium-dependent manner (Green et al., 2008; Kato et al., 2008). Inability to genetically ablate CDPK1 has been assumed to be indicative of essentiality to blood stage infection, but has meant that no in vivo role of CDPK1 has been revealed to date, and the functional consequence of motor phosphorylation—if any—remains unknown. Sebastian et al. (2012) present insight into an in vivo

role of CDPK1. In doing so, they suggest that any activity of CDPK1 in actomyosin motor regulation is likely only one part of a much more interesting story.

To specifically investigate the role of CDPK1 in motile ookinetes, Sebastian et al. (2012) exchanged the native promoter of this kinase in the murine malaria parasite *P. berghei* for one only expressed in blood stages. This genetic sleight of hand allowed viable asexual growth while knocking *Pb*CDPK1 expression below detectable levels in gametocytes and mosquito stages. Surprisingly, ookinete motility in the absence of *Pb*CDPK1 could not even be assessed as development from the fertilized zygote was disrupted, resulting in immotile "retort" ookinetes (Sebastian et al., 2012). In delving into this unexpected developmental disruption, Sebastian et al. (2012) compared both the proteomes and transcriptomes of wild-type and *Pb*CDPK1-knockdown ookinetes. Doing so, it was observed that a number of proteins were depleted in CDPK1 knockdowns, but that this depletion was not associated with a change in transcript levels. Interestingly, this group of dysregulated proteins was significantly enriched for those previously described to be regulated by TR during sexual development, and to rely on DOZI or CITH for stabilization and storage. This suggested that *Pb*CDPK1 is vital for activating translation from a subset of stored mRNAs, and that without it these transcripts remain quiescent.

How *Pb*CDPK1 could alleviate repression of transcripts during ookinete development is something of a mystery. Sebastian et al. (2012) confirm previous findings in showing *Pb*CDPK1 localizes to the parasite plasma membrane, but this localization is unlike that of either DOZI or CITH, which generally show a punctate staining pattern through the parasite cytoplasm (Mair et al., 2006, 2010). It is possible that the subset of P granules regulated by *Pb*CDPK1 is targeted to the plasma membrane during ookinete development, and investigation of the localization of *Pb*CDPK1-regulated transcripts using fluorescence in situ hybridization would be very interesting. This of course would only apply if *Pb*CDPK1 directly regulated TR by phosphorylating components of the silencing machinery, but if this were the case the work of Sebastian et al. (2012) could be the tip of the iceberg regarding the involvement of CDPKs in TR. *Plasmodium* CDPKs have diverse subcellular localizations, and interrogating them for roles in sexual development could reveal involvement in regulating spatially defined subsets of repressed transcripts.

It is also intriguing to note that a number of proteins dysregulated by knockdown of *Pb*CDPK1 are involved in parasite motility and are normally targeted to the parasite periphery (ie. MyoA and MTIP), meaning they colocalize with CDPK1 (Green et al., 2008). Perhaps quiescent mRNAs are often translated at the site their protein products are needed? If TR is indeed important for prompt ookinete development to hasten escape from the midgut, translation of proteins proximal to their localization could be an efficient means of preparing parasites as quickly as possible. It would be enlightening to investigate the importance of *Pb*CDPK1's membrane targeting to its role in TR, by attempting to complement the CDPK1-knockdown phenotype with copies mutated in the N-terminal sequence known to effect its localization.

The smoking gun of a hypothesis for direct regulation of TR by CDPKs could be if DOZI, CITH, or any other P granule components are phosphorylated in a calcium-dependent manner. At this stage however, dysregulation of repressed mRNAs in the absence of *Pb*CDPK1 could be caused simply by a defect upstream in calcium signaling. Clearly, identification of the substrates of *Pb*CDPK1 during ookinete development is now key and should be possible by adapting a chemical genetic approach followed by substrate capture, developed by the Shokat group (Blethrow et al., 2008).

Considering these interesting findings it should be stressed that, since the arrest of *Pb*CDPK1-knockdown parasites at the retort stage prevented an interrogation of subsequent roles in mosquito infection, this work cannot discount involvement of CDPK1 in ookinete motility and midgut traversal, as previously hypothesized. However, it does indicate that any future investigations of CDPK1's role in host-cell invasion and motility will need to remain holistic in their scope or risk capturing only a glimpse of the truth.

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